SUPPLEMENTAL FIGURES

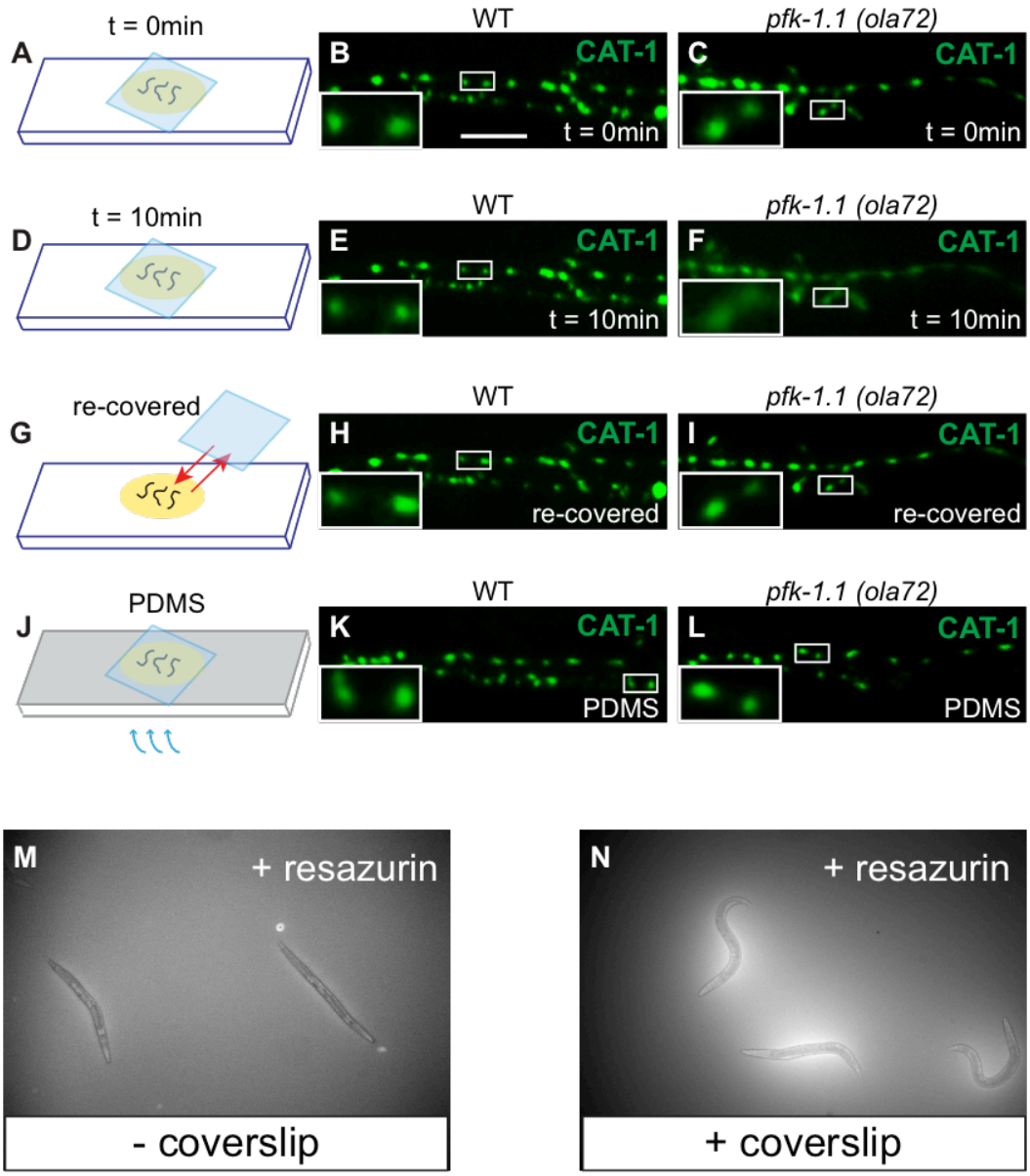

Figure S1, related to Figure 1. *pfk-1.1(ola72)* fails to maintain synaptic vesicle protein clusters under hypoxic conditions

(A-L) Synaptic vesicle marker VMAT/CAT-1 in NSM of wild-type (B, E, H, and K) or *pfk-1.1(ola72)* mutants (C, F, I, and L) mounted as depicted in the cartoon diagrams (A, D, G, and J). Namely, on glass slides and immediately imaged (t = 0min), which is reflective of normoxic

state (A-C); after ten minutes (t = 10min) (D-F); immediately imaged after the glass coverslip was removed for ten seconds (and animals were briefly exposed to normoxic conditions) and replaced over the samples (G-I); imaged after the animals had been mounted for ten minutes using a polydimethylsiloxane (PDMS) slide instead of a glass slide (J-L). PDMS is a gas-permeable silicon-based material (Aoki, 1999). Note that the synaptic vesicle proteins become diffusely localized in *pfk-1.1(ola72)* after ten minutes under the coverslip and then fully recover after seconds of normoxic conditions. Also, *pfk-1.1(ola72)* animals do not express the phenotype when mounted on oxygen-permeable PDMS slides. Insets correspond to zoomed-in (3x) images of the indicated regions.

(M-N) Images of worms taken after 10 minutes of incubation with the redox indicator (resazurin) in the absence (M); or presence (N) of a glass coverslip. Resazurin, when reduced to resorufin, fluoresces under yellow-green light (O'Brien et al., 2000). In presence of a coverslip, there is an accumulation of fluorescence (N), indicating that the animals are experiencing a reduced (hypoxic) environment (note that exposure was increased in M to show the worms, otherwise the image would have been entirely dark, as there was no detectable fluorescence signal in this panel). These observations are consistent with former studies that demonstrated that mounting living cells between a glass slide and a glass cover slip results in hypoxic conditions (Pitts and Toombs, 2004).

As described in the *Supplemental Experimental Procedures*, the original screen that resulted in the identification of *ola72* was designed to identify mutants in genes required for synaptogenesis in the serotonergic neurons, NSM. We screened through 2000 haploid genomes and identified 13 alleles with defects in synaptic patterning. These mutants fall into four categories: (1) Synaptic vesicle signal is diffusely distributed throughout neurites (6 mutants); (2) Synaptic vesicle signal

is very dim or completely absent (4 mutants); (3) Synaptic varicosities appear larger than average (2 mutant); (4) Synaptic vesicle signal appears wild-type upon first examination; after extended exposure to hypoxic conditions under a coverslip (Pitts and Toombs, 2004) the synaptic vesicles became diffusely distributed. This fourth category is the mutant class *pfk-1.1(ola72)* belongs to.

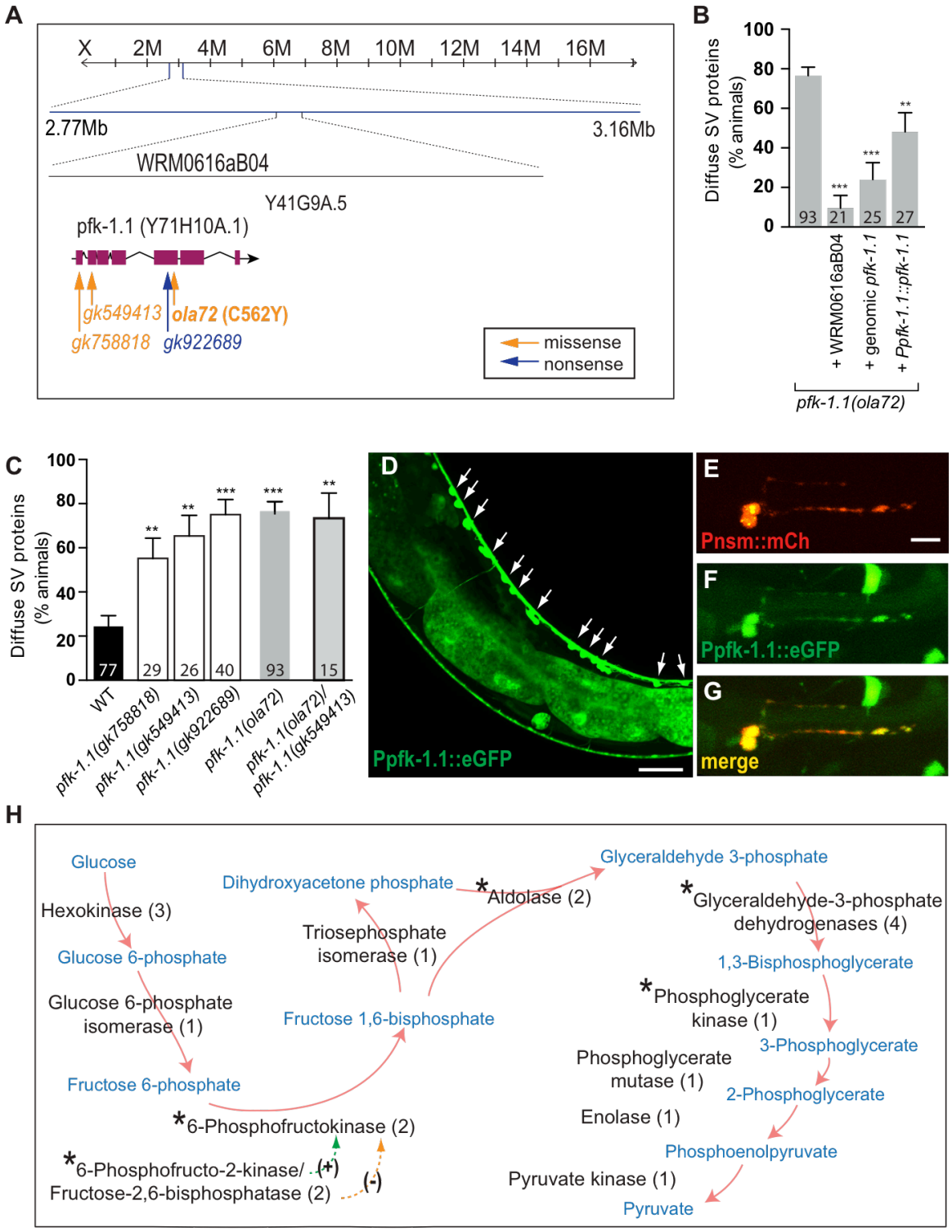

Figure S2, related to Figure 1and Figure 2. Genetic lesions in *pfk-1.1* mutants and endogenous expression pattern

(A-B) Schematic of the genomic region of *pfk-1.1* and locations of the genetic lesions of the *pfk-1.1* alleles examined in this study (marked with arrows) (A); and percentage of animals displaying a diffuse distribution of synaptic vesicle proteins in rescuing backgrounds (B). Single Nucleotide Polymorphism (SNP) mapping was performed and the *ola72* lesion was determined to be located between 2.77 Mb and 3.16 Mb on chromosome X (A). Fosmid WRM0616aB04, which contains the *pfk-1.1* genomic region (as well as un-named gene, *Y41G9A.5*), was shown to rescue the diffuse distribution of synaptic vesicle protein phenotype in *pfk-1.1(ola72)* mutant animals exposed to 10 minutes of hypoxic conditions (induced by mounting on glass slide with coverslip (Pitts and Toombs, 2004)) (B). The *pfk-1.1* promoter driving *pfk-1.1* cDNA also rescues the synaptic vesicle clustering phenotype in *pfk-1.1(ola72)* mutant animals exposed to 10 minutes of hypoxic conditions (B). Number of animals scored is indicated at the bottom of each column. *pfk-1.1(ola72)* is the same as shown in Figure 1J for (B).

(C) Percentage of animals displaying a diffuse distribution of synaptic vesicle proteins after 10 minutes of hypoxic treatment (induced by mounting on glass slide with coverslip (Pitts and Toombs, 2004)). Note that all three independent *pfk-1.1* alleles phenocopy *pfk-1.1(ola72)*, and that *pfk-1.1(gk549413)* fails to complement *pfk-1.1(ola72)*. Number of animals scored is indicated at the bottom of each column. Wild-type control and *pfk-1.1 (ola72)* is the same as shown in Figure 1J.

(D-G) Expression of the *pfk-1.1* promoter is observed in neurons of the ventral and dorsal nerve cords (arrows) (D); and in head neurons, including the NSM neurons (E-G).

(H) Schematic of the glycolysis pathway and mutants examined in this study. Enzymes are shown in black, while substrates are in blue. Numbers inside parentheses indicate the number of identified *C. elegans* homologs for each given enzyme in the pathway. Solid red arrow denotes

enzymatic reactions in the glycolysis pathway. Dashed arrows denote regulatory steps: (+) for activation and (-) for inhibition. Asterisk marks genes examined in this study. We selected the specific enzymes for analyses based on availability of identified alleles from the *Caenorhabditis Genomic Center* (CGC). With the exception of *6-phosphofructokinase/pfk-1.1*, which was identified in our forward genetic screen, all other enzymes were selected based on the availability of deletion alleles.

Scale bar represents 25μm for (D), and 5μm for (E). Error bars denote SEM. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$ between indicated groups.

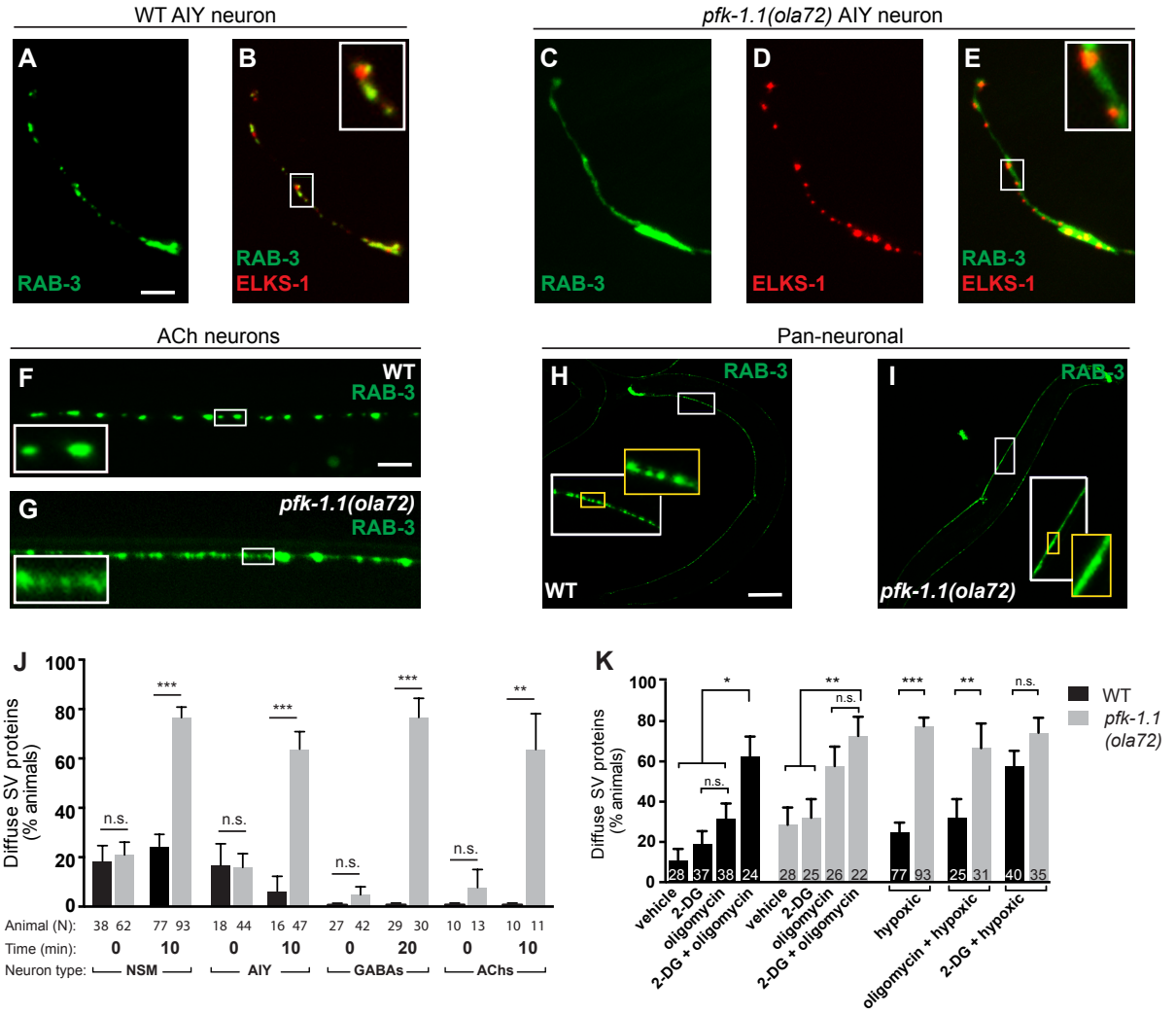

Figure S3, related to Figure1. Synaptic phenotypes in *pfk-1.1(ola72)* mutants under hypoxic conditions.

(A-E) Simultaneous visualization of synaptic vesicle clusters imaged with GFP::RAB-3 (A, C) and active zones imaged with ELKS-1::mCherry (B, D) in AIY neurons of wild-type (A, B); or *pfk-1.1(ola72)* mutant animals (C-E) mounted on glass slides and exposed to hypoxic conditions for 10 minutes (Pitts and Toombs, 2004). Note that in *pfk-1.1(ola72)* mutant animals, synaptic vesicle clusters become diffusely distributed (C); as compared to wild-type animals under similar conditions (A). Also note that active zone proteins remain punctate in both wild-type (B); and

pfk-1.1(ola72) mutant backgrounds (D, E). Insets correspond to zoomed-in (3x) images of the indicated regions.

(F-G) VENUS::RAB-3 in dorsal acetylcholine motor neurons of wild-type (F); and *pfk-1.1(ola72*) animals (G) mounted on glass slides and exposed to hypoxic conditions for 20 minutes (Pitts and Toombs, 2004). Insets correspond to zoomed-in (3x) images of the indicated regions.

(H-I) Pan-neuronal expression of GFP::RAB-3 in wild-type (H); and *pfk-1.1(ola72*) mutant animals (I) after being mounted on glass slides and exposed to hypoxic conditions for 20 minutes. Each inset (white and yellow outlined box) is of the ventral nerve cord and 3x magnified from corresponding regions indicated in the images.

(J) Percentage of animals displaying a diffuse distribution of synaptic vesicle proteins after 10 (or 20 minutes, as indicated) of exposure to hypoxic conditions (induced by mounting on glass slide with a glass coverslip (Pitts and Toombs, 2004); also refer to Figure S1). At 0 minutes, no differences are observed between the vesicle clusters of neurons in wild-type (black bars) and *pfk-1.1(ola72)* mutant animals (gray bars) for the four different types of neurons examined. By 10 (or 20) minutes, a significant percentage of *pfk-1.1(ola72)* mutant animals exhibit pronounced abnormal distribution of synaptic vesicle protein phenotypes in all neurons examined, but not the wild-type animals. NSM wild-type and *pfk-1.1(ola72)* are the same as shown in Figure 1J.

(K) Percentage of animals displaying a diffuse distribution of synaptic vesicle proteins in wild-type (black bars) or *pfk-1.1 (ola72)* mutant animals (gray bars) under varying conditions. In the first eight columns, animals under normoxic conditions were treated for 10 minutes with pharmacological agents as indicated in the graph and described in the *Supplemental Experimental Procedures*. Note that in wild-type animals, inhibition of oxidative

phosphorylation (with 1uM oligomycin) and of glycolysis (with 10uM 2-deoxy-D-glucose, indicated as 2-DG) have only partial effects on the percentage of animals displaying diffuse synaptic vesicle (SV) clusters, while simultaneous inhibition of glycolysis and oxidative phosphorylation results in an enhancement of the phenotype. In *pfk-1.1(ola72)* animals, oligomycin, but not 2-DG, enhances the phenotype, suggesting that *pfk-1.1(ola72)* acts in the same pathway as 2-DG inhibition, namely, glycolysis. Note that in the last six columns of the graph, hypoxic conditions do not enhance the oligomycin effect for wild-type animals, but do enhance the 2-DG effect. Together, these analyses uncover a cooperative role between oxidative phosphorylation and glycolysis in maintaining the synaptic vesicle pattern in neurons, and indicate that under conditions of energy stress in which the activity of the oxidative phosphorylation pathway is decreased, the glycolytic pathway is required for the maintenance of synaptic vesicle clusters *in vivo.* Vehicle (or normoxic) and hypoxic are the same as shown in Figure 1J.

Scale bar represents 5μm; except for (H, I), in which scale bar represents 100μm. Error bars denote SEM. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$ between indicated groups.

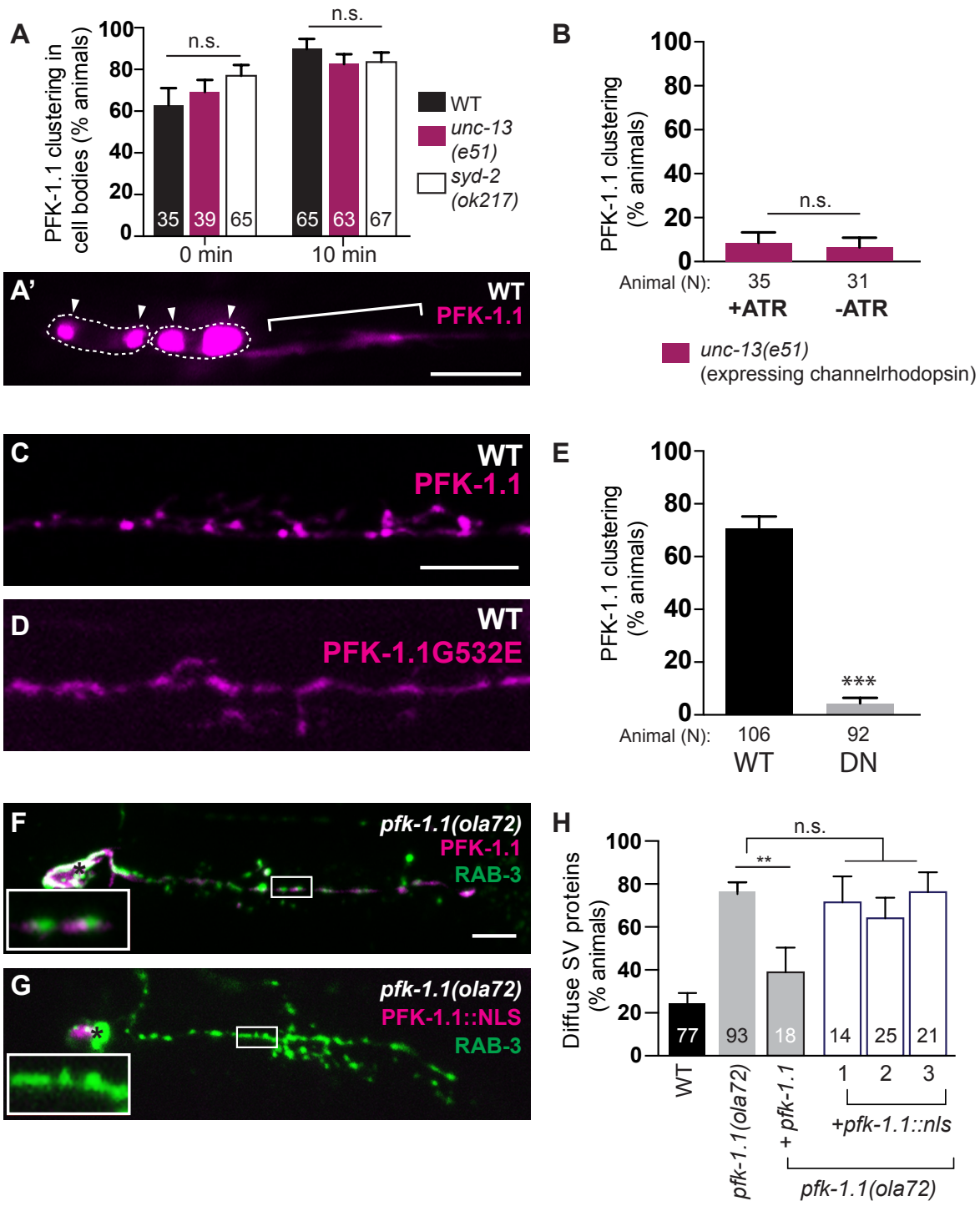

Figure S4, related to Figure 5 and Figure 7. Localization of PFK-1.1 to presynaptic sites is important for its function in sustaining the synaptic vesicle cycle.

(A-A') Percentage of animals displaying PFK-1.1 clusters in the cell bodies of GABA neurons in wild-type (black bar), *unc-13(e51)* (red bar), and *syd-2(ok217)* (white bar) mutant animals immediately after mounting them under the coverslip (0 min) or after 10 minutes (10 min), causing hypoxic conditions (A). Even before hypoxic conditions ensue, PFK-1.1 (pseudocolored magenta) is observed as clustered (arrowheads) in the cell bodies (outlined by dotted lines) of wild-type animal, while the neurites (bracket) largely show a diffuse distribution of the PFK-1.1 (A').

(B) Percentage of *unc-13(e51)* mutant animals displaying PFK-1.1 clusters in the GABA neurons of *oxIs352* animals (expressing channelrhodopsin cell-specifically in GABA neurons) and stimulated with blue light in the absence (-ATR) or presence (+ATR) of rhodopsin co-factor all-trans-retinal (ATR). PFK-1.1 clustering is suppressed even in the presence of ATR, or neuronal stimulation, in *unc-13* mutants. Number of animals tested is indicated at the bottom of each column.

(C-E) PFK-1.1 (pseudocolored magenta) forms clusters under hypoxia (C). The dominant negative (DN) form, PFK-1.1(G532E), fails to form clusters under the same condition (D). Percentage of animals displaying PFK-1.1 (WT) or PFK-1.1(G532E) (DN) clusters under hypoxic conditions (E). Number of animals scored is indicated at the bottom of each column for (E). Wild-type control is the same as shown in Figure 5G for (E).

(F) PFK-1.1::eGFP (pseudocolored magenta) clusters to presynaptic sites when expressed in the NSM neurons of *pfk-1.1(ola72)* animals and rescues the clustering of the synaptic vesicle protein RAB-3 under hypoxic conditions. Cell body is indicated by asterisk.

(G) PFK-1.1::eGFP::NLS (pseudocolored magenta) localizes to the nucleus, is not observed at synapses when expressed in the NSM neurons of *pfk-1.1(ola72)* animals and fails to rescue the

clustering of RAB-3 under hypoxic conditions (quantified for three independent transgenic lines in (H)). Cell body is indicated by asterisk.

(H) Percentage of animals displaying abnormal distribution of synaptic vesicle proteins under hypoxic conditions in wild-type (black bar), *pfk-1.1(ola72)* (gray bar), *pfk-1.1(ola72)* expressing a rescuing construct PFK-1.1::eGFP cell-specifically in NSM (gray bar with black outline), and three independent transgenic lines of *pfk-1.1(ola72)* mutant animals expressing PFK-1.1::eGFP::NLS cell-specifically in NSM (white bars). Number of animals scored is indicated at the bottom of each column. Wild-type control and *pfk-1.1(ola72)* are the same as shown in Figure 1J.

Scale bar represents 5μm. Error bars denote SEM. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$ between indicated groups.

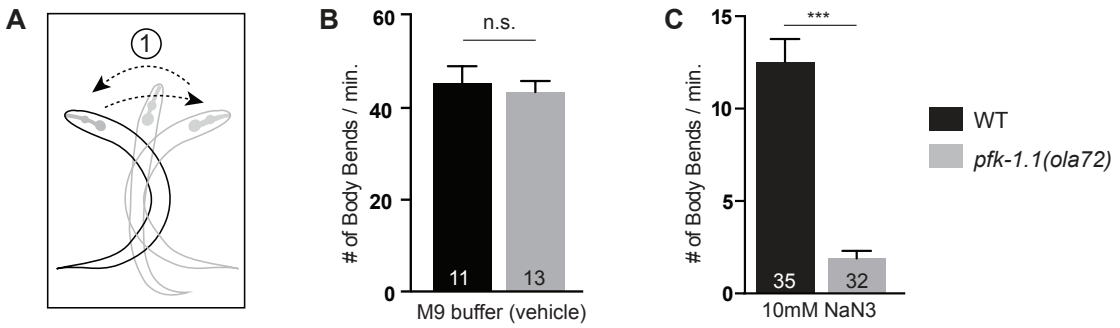

Figure S5, related to Figure 8. *pfk-1.1* mutants have impaired locomotion compared to wild-types upon inhibition of oxidative phosphorylation.

(A) Cartoon illustration of one full body bend in *C. elegans* as indicated by the arrows.

(B-C) Number of body bends measured for 1 minute after a four-minute bath in M9 buffer alone (B) or in a 10mM sodium azide (NaN_3) (C). M9 buffer alone produced no difference in the number of body bends between wild-type and *pfk-1.1(ola72)* mutant animals while *pfk-1.1(ola72)* (B) mutants showed significant decrease in the number of body bends compared to that of the wild-type under sodium azide treatment (C). Number of animals scored is indicated at the bottom of each column.

SUPPLEMENTAL MOVIES

Movie S1, related to Figure 1. Time lapse movie of synaptobrevin/SNB-1::GFP in GABA neuron of *pfk-1.1(ola72)* mutant animal under hypoxic conditions.

Images acquired at one minute interval from 5 to 20 minutes during hypoxic treatment. Corresponds to Figures 1M and 1N.

Movie S2, related to Figure 4. Time lapse movie of PFK-1.1::eGFP (pseudo colored magenta) localization in NSM neuron in wild-type animal under hypoxic conditions.

Images acquired at one minute interval from approximately 10 minutes to 30 minutes during hypoxic treatment. Correspond to Figures 4A and 4A'.

SUPPLEMENTAL EXPREIMENTAL PROCEDURES

Strains and genetics

Worms were raised on nematode growth media plates at 20°C using OP50 *Escherichia coli* as a food source (Brenner, 1974). For wild-type nematodes, *C. elegans* Bristol strain N2 was used. The following mutant strains were obtained through the *Caenorhabditis* Genetics Center: *juIs1[Punc-25::snb-1::gfp; lin-15(+)], jsIs682[Prab-3::gfp::rab-3], pfk-1.1(gk549413), pfk-1.1(gk758818), pfk-1.1(gk922689), pfkb-1.1(ok2733), gpd-3(ok2870), unc-57(ok310), unc-11(e47), unc-26(s1710), unc-13(e450), unc-13(e51), unc-104(e1265), klp-6(sy511), ric-7(nu447), syd-2(ju37), syd-2(ok217),* and *unc-49(e407). aldo-1(tm5782)* and *pgk-1(tm5613)* were received from Shohei Mitani (Tokyo Women's Medical University, Tokyo, Japan). *nuIs168[Pmyo-2::gfp; Punc-129::Venus::rab-3]* was provided by Jihong Bai (Fred Hutchinson Cancer Research Center, Seattle, Washington). *lev-8(ok1519)* was provided by Michael Koelle (Yale University, New Haven, Connecticut). *drp-1(tm408)* was provided by Marc Hammarlund (Yale University, New Haven, Connecticut). *syd-2(ju37); juIs330 [Punc-25::syd-2]* was provided by Mei Zhen (University of Toronto, Canada). Other strains used in the study are as follows: *olaIs1 [Ptph-1::mCherry; Ptph-1::cat-1::gfp], WyEx505 [Pttx-3::mCherry::erc; Pttx-3::GFP::rab-3]*, *oxSi91[Punc-17::ChIEF::mCherry]*, and *oxIs352 [Punc-47::ChR2::mCherry; lin-15(+)]*.

Molecular biology and transgenic lines

Expression clones were generated using the Gateway system (Invitrogen). Detailed cloning information will be provided upon request. Transgenic strains (0.5–30 ng/µl) were generated using standard techniques (Mello and Fire, 1995) and coinjected with markers *Punc-122::gfp* or *Punc-122::rfp*. The following strains were generated: *olaEx1556 [WRM016aB04; Pttx-*

3::mCherry], olaEx2337 [Ppfk-1.1::pfk-1.1], olaEx1923 [Ppfk-1.1::pfk-1.1], olaEx1920 [Ptph-1::pfk-1.1], olaEx2085 [Ppfk-1.1::egfp], olaEx1641 [Ppfk-1.1::egfp; Ptph-1::mCherry], olaEx2014 [Ptph-1::pfk-1.1::egfp; Ptph-1:mCherry], olaEx2016 [Ptph-1::pfk-1.1::egfp; Ptph-1::mCherry::rab-3], olaEx2209 [Ptph-1::pfk-1.1::egfp; Ptph-1::mCherry::rab-3], olaEx2241 [Ptph-1::pfk-1.1::mCherry; Ptph-1::aldo-1::egfp], olaEx2249 [Ptph-1::pfk-1.1::mCherry; Ptph-1::gpd-3::egfp], olaEx2274 [Punc-47::pfk-1.1::egfp], olaEx2245 [Pttx-3:sl2:pfk-1.1:egfp; *Pttx-3:mCh:rab-3]*, *olaEx2544 [Ptph-1::pfk-1.1G532E::egfp; Ptph-1::mCherry::rab-3], olaEx2545 [Ptph-1::pfk-1.1G532E]*, *olaEx2330 [Ptph-1::pfk-1.1::egfp::NLS; Ptph-1:mCherry:rab-3]*, *olaEx2290 [Ptph-1::pfk-1.1::egfp::NLS; Ptph-1:mCherry:rab-3]*, *olaEx2291 [Ptph-1::pfk-1.1::egfp::NLS; Ptph-1:mCherry:rab-3]*, and *olaex2671 [Ptph-1::mCherry::rab-3]*.

Screen and positional cloning

Worms expressing CAT-1::GFP and cytosolic mCherry in NSM neuron (*olaIs1*) were mutagenized with ethyl methanesulfonate (EMS) as described previously (Brenner, 1974). The original screen was designed to identify mutants in genes required for synaptogenesis in the serotonergic neurons (NSM). The screen was performed as previously described (Colon-Ramos et al., 2007; Jin, 2005; Schaefer et al., 2000; Shao et al., 2013; Shen and Bargmann, 2003; Sieburth et al., 2005; Yeh et al., 2005). Briefly, F_1 progeny of mutagenized P_0 worms were cloned onto individual plates, and F_2 progeny were screened using a Leica DM500B compound fluorescent microscope to visualize the distribution of the synaptic vesicular monoamine transporter (VMAT), CAT-1::GFP. NSM neurons were first inspected for appropriate cellular morphology and position, as well as axon and dendrite extension and arborization (by using the cytoplasmic mCherry marker). Next, the position, number, intensity, and distribution of the synaptic vesicle signal was assessed using our CAT-1::GFP marker. We screened through 2000

haploid genomes and identified 13 alleles with defects in synaptic patterning. These mutants fall into four categories: (1) Synaptic vesicle signal is diffusely distributed throughout neurites (6 mutants); (2) Synaptic vesicle signal is very dim or completely absent (4 mutants); (3) Synaptic varicosities appear larger than average (2 mutant); (4) Synaptic vesicle signal appears wild-type upon first examination; after extended exposure to hypoxic conditions under a coverslip (Pitts and Toombs, 2004) the synaptic vesicles became diffusely distributed. This fourth category is the mutant class *pfk-1.1(ola72)* belongs to.

The *ola72* allele was mapped to a 0.39Mbp region on chromosome X using single nucleotide polymorphisms and the CB4856 strain as described (Davis et al., 2005). Fourteen fosmids that cover this region were injected into *ola72* mutants and examined for rescue of synaptic vesicle clustering defects. *ola72/pfk-1.1* trans-heterozygotes were examined for complementation. Sanger sequencing was performed to identify the genetic lesion in the *ola72* allele.

Cell autonomy and rescue of *ola72*

The *ola72* mutant phenotype was rescued by both the full *pfk-1.1* genomic sequence and *pfk-1.1* cDNA under the regulation of its endogenous promoter (fragment 1.5kb upstream of *pfk-1.1*). Cell-specific rescue in NSM was achieved with a plasmid driving the expression of *pfk-1.1* cDNA under the NSM-specific *tph-1* promoter fragment as described (Nelson and Colón-Ramos, 2013). *pfk-1.1* cDNA tagged with eGFP at its C-terminus under the NSM-specific promoter also rescued the *ola72* mutant phenotype (Figure S4H).

Inducing hypoxia with glass coverslips and slides

Glass coverslips have been used to induce hypoxia in cell cultures (Pitts and Toombs, 2004). The reduced environment generated by mounting 10-15 live worms on glass slides was examined by

using redox indicator resazurin (25ug/mL) dissolved in water. Resazurin when reduced to resorufin gives off fluorescence under yellow-green light (O'Brien et al., 2000). Using resazurin, we confirmed that worms between a glass coverslip and slide experience reduced, or hypoxic, conditions; Leica DM500B compound fluorescent microscope was used to acquire the images (Figures S1M and S1N). Gas permeable slides were made with Sylgard-184 (polydimethylsiloxane, or PDMS) (Dow Corning) according to manufacturer instructions.

Inhibiting oxidative phosphorylation or glycolysis using a hypoxia chamber or pharmacological treatments

Hypoxia assays were performed using a chamber (similar to Billups-Rothernberg modular incubator chamber MIC-101) generously shared by Dr. Americo Esquibies (Yale University School of Medicine). Worms were mounted on a slide, placed in a hypoxia chamber and flushed for 4 minutes with nitrogen gas. The chamber was sealed and the worms were exposed to hypoxic conditions for 10 minutes. After the exposure, phenotypes were scored immediately under a Leica DM500B compound microscope. For carbon dioxide exposure, a smaller chamber was built using glass bottom culture dish (MatTek). Worms were placed in the culture dish and exposed to carbon dioxide for 10 minutes, and scored immediately. For pharmacological disruption of oxidative phosphorylation or glycolysis, worms were mounted on a slide with 10mM sodium azide (NaN_3), 10uM of 2-deoxy-D-glucose (2-DG), or 1uM of oligomycin dissolved in muscimol, and scored after 10 minutes.

Neuronal stimulation with pharmacological treatments or optogenetics.

To pharmacologically stimulate GABA neurons, worms were mounted on a slide in 1mM levamisole (Sigma) and imaged immediately in spinning-disc confocal microscope (PerkinElmer Life and Analytical Sciences). As a control, 50mM of muscimol (Abcam) was used. To

optogenetically stimulate GABA neurons, a strain expressing channelrhodopsin 2 in GABA neurons (*oxIs352*) (Liu et al., 2009) was used. All-trans-retinal and OP50-seeded plates were prepared as described previously (Liu et al., 2009). Briefly, 4uL of 100uM all-trans-retinal was added to 200uL of OP50 culture to seed each plate. Channelrhodopsin expressing worms were grown in all-trans-retinal seeded plate for minimum of 16 hours before stimulation. Worms were then mounted to a gas permeable PDMS slide set up, immobilized in muscimol, exposed to blue light for 5 minutes using a Leica DM500B compound microscope (approximately 0.6mW/mm^2), and examined for formation of PFK-1.1 clusters. As a control, the same strains were grown without all-trans-retinal, and tested under the same conditions.

Electrophysiology

Worms expressing the channelrhodopsin I/II chimera ChIEF in acetylcholine neurons (*oxSi91*) were dissected and patch-clamp physiology was conducted as previously described (Richmond et al., 1999; Richmond and Jorgensen, 1999). For oligomycin experiments, the dissected preps were exposed to 1uM oligomycin for 5 minutes prior to patch clamp recordings. The stimulation protocol included a pre-pulse followed by a 5-second recovery period and 30-second stimulation train (10Hz). Test pulses were delivered at increasing time increments and normalized to the first spike in the train to assess recovery. Two test-pulses were delivered per animal at ≥15 seconds apart. By analyzing the overlap of the first and second pulses at intermediate time-points, we determined that a single test-pulse did not affect the amplitude of the second pulse. For repeated stimulus trains, the full protocol was repeated with 70 seconds between the end of the first train and the beginning of the following train. Peak current was measured for each evoked response (red Xs in Figure 8B) and normalized to the amplitude of the first pulse of the stimulus train. The evoked amplitudes were plotted vs. time.

Behavioral experiments

C. elegans nematodes move in a wave-like fashion by executing dorsal-ventral body bends, and this sinusoidal body motion can be observed both on solid substrates and when worms are placed in an aqueous environment (Ghosh and Emmons, 2008). To examine how the locomotion of these animals is affected under hypoxic conditions, an Anaerobe Pouch System (Becton Dickinson & Co) was used to induce hypoxia. Animals were placed in M9 solution on slides, which were then placed inside the gas-impermeable pouch along with a wet paper towel and anaerobe gas-generating sachet. To examine how the locomotion of animals is affected under conditions of energetic stress, animals were placed in 10mM sodium azide (NaN_3) solution dissolved in M9 on top of a slide. After 4 minutes of acclimation, the number of body bends was scored for 1 minute under a dissection scope. Bending of the head of the worm to one side and back to the initial side was counted as one body bend as illustrated in Figure 8H. M9 solution was used as a control and, as expected, no difference in the number of body bends between wild-type and *pfk-1.1* mutant animals were observed in this control (data not shown). *p values* were calculated using the Mann-Whitney *U* test.

Microscopy, FRAP, and imaging

Images of fluorescently tagged fusion proteins were captured in live *C. elegans* nematodes using a 60 CFI Plan Apo VC, numerical aperture 1.4, oil-immersion objective on an UltraView VoX spinning-disc confocal microscope (PerkinElmer Life and Analytical Sciences). Worms were immobilized using 50mM muscimol (Abcam). Image J and Photoshop were used to analyze images, which were oriented anterior to the left and dorsal up. Maximum projections were used for all the confocal images, unless otherwise stated. Ratiometric images were generated by using the Volocity software (Perkin Elmer). For the Fluorescence Recovery After Photobleaching

(FRAP) experiment, the spinning-disc confocal microscope and Volocity FRAP Plugin (Perkin Elmer) were used. Single plane images were acquired at 3-second intervals post-photobleaching. Acquired data was normalized using FRAP analysis in Volocity and fitted to a single exponential curve. For FRAP analysis, *p values* were calculated using the Mann-Whitney *U* test.

Quantification of phenotypic expressivity

To quantify synaptic enrichment of the indicated proteins (synaptic vesicle proteins or PFK-1.1), fluorescence values for individual neurites (ventral neurite for the NSM neuron, Zone3 for the AIY neuron, and dorsal and ventral neurite for GABA neurons) were obtained through segmented line scans using ImageJ. A sliding window of 2µm was used to identify all the local fluorescence peak values and trough values for an individual neuron (the maximum and the minimum fluorescence values in a 2µm interval, respectively). Synaptic enrichment was then calculated as % $\Delta F/F$ as previously described (Bai et al., 2010; Dittman and Kaplan, 2006). Briefly, all the identified local maximum and minimum fluorescence values in a given neurite (local F_{peak} and local F_{trough}) were averaged and used to calculate % $\Delta F/F$, with % $\Delta F/F$ being the percent difference between average peak-to-trough fluorescence (F) defined as 100 x ($F_{peak} - F_{trough}$)/F_{trough}. All the images used in the quantification analyses were obtained using identical microscopy settings. For the quantification of the average number of PFK-1.1 puncta in AIY neurons, confocal images of AIY neurons were analyzed and scored for PFK-1.1 puncta in synaptic and asynaptic regions (Colon-Ramos et al., 2007; White et al., 1986). Statistical analyses were performed with Prism (GraphPad) and *p values* were calculated using the Mann-Whitney *U* test.

Quantification of phenotypic penetrance

Animals were scored as displaying either “punctate” or “diffuse” phenotypes for synaptic vesicles proteins or PFK-1.1 after specified manipulations. “Punctate” or “diffuse” phenotypes were first qualitatively defined for the purposes of the genetic screens. To provide a quantitative definition that described the phenotypes we saw, we present what was meant by “diffuse” based on DF/F values. Briefly, we scored the distribution of synaptic vesicle proteins in wild-type or mutant animals by performing line scans, and calculated the DF/F for wild-type (N=16) and *pfk-1.1(ola72)* mutants (N=19). The average value for wild-type animals is 5.17 DF/F (fold increase of peak-to-trough fluorescence), while the average value for *pfk-1.1(ola72)* mutants is 2.94 DF/F fold. We then defined the “diffuse” distribution of vesicle clusters based on this average value observed for *pfk-1.1(ola72)* mutants and established a threshold at 3 DF/F for identifying an animal as either “diffuse” or “punctate”. Calculations, using this value, of the number of animals with a “diffuse” distribution of vesicle clusters reveal that approximately 20% of wild-type animals vs. 60% of *pfk-1.1(ola72)* mutant animals display a diffuse clustering phenotype under hypoxic conditions. We used these parameters as guidelines for scoring the phenotypic penetrance of all conditions and genotypes described in this study. We validated the approach by qualitatively scoring blindly the percentage of animals that exhibit a diffuse distribution of synaptic vesicle proteins for wild-type and for *pfk-1.1(ola72)* animals. We determined that the number we obtained after qualitative assessments of the phenotype and blind scoring were similar to the ones which were generated by measuring synaptic distribution of vesicular proteins using line scans, calculating the DF/F signal and then scoring individuals animals based on the 3 DF/F threshold definition of “diffuse”. Statistical analyses were performed with Prism (GraphPad) and *p values* were calculated using Fisher’s exact test.

Quantification of colocalization

To quantify the relative distance between two fluorescently tagged proteins in the colocalization experiments, we used ImageJ and line scans of neurites to score the pixel fluorescence values in the green and red channels. The relative location corresponding to the maximum pixel fluorescence values for each channel was then used to calculate the distance between the two fluorescently tagged proteins.

SUPPLEMENTAL REFERENCES

Aoki, T. (1999). Macromolecular design of permselective membranes. Progress in Polymer Science *24*, 951-993.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71-94.

Davis, M.W., Hammarlund, M., Harrach, T., Hullett, P., Olsen, S., and Jorgensen, E.M. (2005). Rapid single nucleotide polymorphism mapping in C. elegans. BMC genomics *6*, 118.

Ghosh, R., and Emmons, S.W. (2008). Episodic swimming behavior in the nematode C. elegans. The Journal of experimental biology *211*, 3703-3711.

Jin, Y. (2005). Synaptogenesis. WormBook : the online review of C elegans biology, 1-11.

Mello, C., and Fire, A. (1995). DNA transformation. Methods in cell biology *48*, 451-482.

Schaefer, A.M., Hadwiger, G.D., and Nonet, M.L. (2000). rpm-1, a conserved neuronal gene that regulates targeting and synaptogenesis in C. elegans. Neuron *26*, 345-356.

Shao, Z., Watanabe, S., Christensen, R., Jorgensen, E.M., and Colon-Ramos, D.A. (2013). Synapse location during growth depends on glia location. Cell *154*, 337-350.

Shen, K., and Bargmann, C.I. (2003). The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. elegans. Cell *112*, 619-630.

Sieburth, D., Ch'ng, Q., Dybbs, M., Tavazoie, M., Kennedy, S., Wang, D., Dupuy, D., Rual, J.F., Hill, D.E., Vidal, M*., et al.* (2005). Systematic analysis of genes required for synapse structure and function. Nature *436*, 510-517.

Yeh, E., Kawano, T., Weimer, R.M., Bessereau, J.L., and Zhen, M. (2005). Identification of genes involved in synaptogenesis using a fluorescent active zone marker in Caenorhabditis elegans. The Journal of neuroscience : the official journal of the Society for Neuroscience *25*, 3833-3841.